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Radiosynthesis and Biological Distribution of ^{18}F-Labeled Perfluorinated Alkyl Substances

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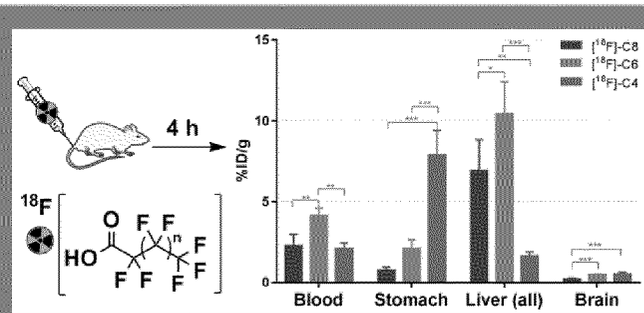
* Supporting Information

A novel method for radiolabeling perfluorinated alkyl substances (PFAS) with fluorine 18 has been developed, and after purification, the stability and biological distribution in healthy mice were evaluated. Three PFAS, [^{18}F]PFOA (C8), [^{18}F]PFHxA (C6), and [^{18}F]PFBA (C4), were readily labeled and isolated in average yields between 12 and 31%. The stability of each compound was monitored in 0.1% ammonium hydroxide (NH_4OH) in methanol, in saline, and in human, mouse, and rat sera. The amount of intact, radiolabeled PFAS was determined by radiometric instant thin layer chromatography and was calculated by the amount of free fluorine 18 observed over time. All compounds were highly stable in 0.1% NH_4OH in methanol and saline, with <10% defluorination observed after 4 h. Interestingly, each compound had differing affinities for the serum proteins. In vivo biodistribution studies in mice showed uptake in all organs examined, with the highest uptake being exhibited in the liver for both [^{18}F]PFOA and [^{18}F]PFHxA and the stomach for [^{18}F]PFBA. The results of this initial study suggest that this method could be valuable in helping to determine the biological uptake of any PFAS in mammals.



INTRODUCTION

While there is significant literature precedent in which the distribution of varying chain lengths and derivatives of perfluorinated alkyl substances (PFAS) in tissues, both animal[1,2] and human, is discussed,[3−5] this is a difficult assessment to make because of the difficulty of PFAS analysis in biological matrices. PFAS can be difficult to measure analytically in biological systems because analysis traditionally involves isolating the tissues, digestion of the tissue, and extraction of the compounds. It subsequently involves the use of highly sensitive techniques such as high-performance liquid chromatography coupled to tandem mass spectrometry[6−8] or combustion-IC[9] as the PFAS family of compounds has no convenient UV−visible signatures. To make these measurements in living biological systems, one common technique is the use of radioisotopic labeling, but there has been very little published using radiolabeled PFAS to assess biodistribution. To date, the only studies include ^{35}S-labeled perfluorosulfonic acid (PFOS)[10] and ^{14}C-labeled perfluorooctanoic acid (PFOA)[11−13] but no ^{18}F-labeled derivatives. In this paper, we present the first radiolabeling of PFAS with fluorine 18 ($t_{1/2}$ = 110 min), which allows for tracking of the biodistribution of these compounds in real time using positron emission tomography (PET), and precise quantification of the amount of radiolabeled compound inside organs and tissues of interest. The ease with which any PFAS compound could be radiolabeled with $^{18}F^-$ is chemically distinct from the significant organic synthesis techniques required to correctly label PFAS with either ^{35}S or ^{14}C. This technique allows for radiolabeling and subsequent purification in <1 h.

The work presented here focuses on the development of the ^{18}F-labeled derivatives of perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHxA), and perfluorobutanoic acid (PFBA). While any representative PFAS could have been studied, the proof-of-concept study presented here started with three carboxylic acids of varying chain lengths. Via induction of a $^{19}F \rightarrow {}^{18}F$ exchange, the native compound (i.e., without structural modifications) can be studied, and the pharmacokinetic properties of the radiolabeled compounds are identical to those of the native PFAS of interest. Initial studies to determine

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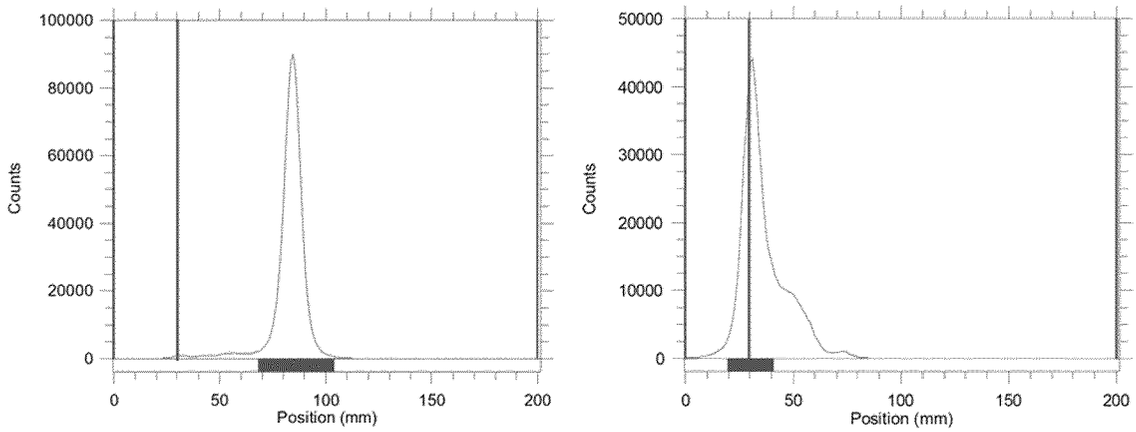

Figure 1. Example of iTLC results for (A) [^{18}F]PFHxA (R_f = 1) and (B) free $^{18}F^-$ (R_f = 0). The origin is marked with a blue line. Instant TLC strips were developed in 0.1% NH_4OH in methanol.

the in vitro stability of the radiolabeled compounds and in vivo biological distribution in healthy mice were also performed.

EXPERIMENTAL SECTION

Radiosynthesis and Isolation of [^{18}F]PFOA, [^{18}F]-PFHxA, and [^{18}F]PFBA. The radiosynthesis and isolation of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA are described in detail in the Supporting Information. In summary, ^{18}O-enriched water was bombarded with a 20 MeV proton beam from the University of Alabama medical cyclotron facility at 40 μA. For the amount of starting activity needed, the target was bombarded for ~10 min. The $^{18}F^-$ produced was extracted from the water target by solid-phase extraction and purified for radiosynthesis. The purity of the [^{18}F]PFAS was determined by the amount of free $^{18}F^-$ in solution as determined by radiometric instant thin layer chromatography. Radiosynthesis and purification of each PFAS took approximately 1 h.

Stability Studies. The stabilities of the radiolabeled PFAS were tested under a variety of conditions: (1) in 0.1% NH_4OH in methanol at room temperature, (2) in saline at room temperature, (3) in mouse serum at 37 °C, (4) in human serum at 37 °C, and (5) in rat serum at 37 °C. For each condition, the amount of intact, radiolabeled PFAS was determined at 30 min, 1 h, 2 h, and 4 h with three replicates. With the exception of the 0.1% NH_4OH in methanol solution (as this was what the [^{18}F]PFAS were isolated in), 25 μL of the desired [^{18}F]PFAS was added to 225 μL of the corresponding solution (saline or sera) in a 1.5 mL Eppendorf microcentrifuge tube and incubated at the temperatures mentioned above. The amount of activity added to tubes was dependent on final isolation yields. For these studies, 7.4 MBq (200 μCi) of [^{18}F]PFOA, 7.03 MBq (190 μCi) of [^{18}F]PHxA, and 3.3 MBq (90 μCi) of [^{18}F]PFBA were used.

In both the NH_4OH and saline studies, a 1 μL aliquot of the solution was removed at each time point and spotted for instant thin layer chromatography (iTLC) analysis (0.1% NH_4OH in the methanol mobile phase). The serum stability studies were performed for each condition twice, using two different methods of analysis. In the first method of analysis, a 1 μL aliquot of the solution was removed for iTLC analysis at each time point. In the second method of analysis, a 50 μL aliquot was taken from each tube and the serum proteins were precipitated with the addition of 150 μL of acetonitrile. The aliquots were vortexed and the proteins pelleted via centrifugation. The supernatant, containing the non-protein-bound compound, was collected, and the pellet was further washed with an additional 150 μL of acetonitrile. The wash was combined with the original supernatant. The combined supernatants (total collected volume of ~300 μL), and the protein pellets were each counted using an automated Wizard^2 2480 gamma counter (PerkinElmer). The data were analyzed and are presented as the percentage (of total) bound to serum proteins. Afterward, the supernatant was also analyzed via iTLC in the same manner as previously outlined.

Biodistribution Studies in Normal Mice. All animal studies were conducted in compliance with the guidelines for the care and use of research animals established by the University of Alabama's Institutional Animal Care and Use Committee. After radiolabeling, each respective [^{18}F]PFAS was diluted into saline to a final concentration of ~0.074 MBq (2 μCi)/μL. Healthy, male CD1 mice (n = 4 per compound) were then intravenously injected with an average of 7.22 MBq (195 μCi), approximately 100 μL, of the desired [^{18}F]PFAS. Animals were anesthetized with 0.1% isofluorane and euthanized 4 h postinjection; organs and tissues of interest were harvested and weighed, and the radioactivity was measured using an automated gamma counter. Data were decay-corrected and calculated as the percent injected dose per gram of tissue (% ID/g).

RESULTS AND DISCUSSION

Radiosynthesis and Isolation of [^{18}F]PFOA, [^{18}F]-PFHxA, and [^{18}F]PFBA. All three PFAS tested were successfully radiolabeled with $^{18}F^-$ and isolated. Initial attempts to synthesize [^{18}F]PFOA were performed in anhydrous acetonitrile, but the radiochemical yields were not consistent. The [^{18}F]PFOA reaction mixture had to be dissolved in ≥500 μL of acetonitrile to account for solvent loss (due to evaporation) during the radiolabeling process. The larger reaction volume also resulted in decreased overall yields that varied widely. The problem with radiolabeling [^{18}F]PFOA was solved by performing the reaction in 300 μL of dimethyl sulfoxide at 125 °C, and thus, this method was used for the two remaining PFAS. Additionally, results from data (not shown) indicated that at lower temperatures (~100 °C) the exchange reaction did not proceed with PFBA or PFHxA.

Previously published work has shown that fluoride (non-radioactive) can be separated and purified from PFAS on WAX

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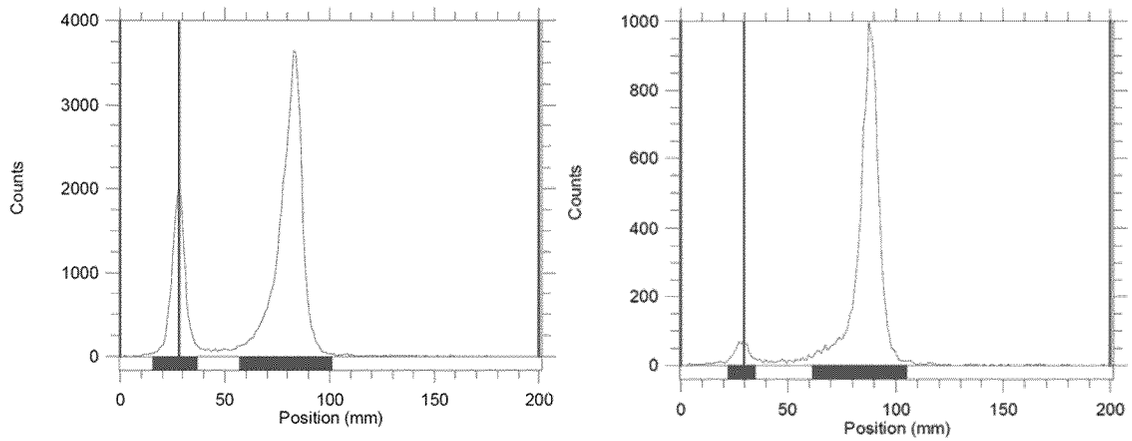

Figure 2. (A) iTLC results for the [^{18}F]PFHxA/rat serum mixture 1 h after the end of synthesis. The R_f of [^{18}F]PFHxA equals 1, and the R_f of [^{18}F]PFHxA with serum equals 0. (B) iTLC results of the acetonitrile supernatant of the same [^{18}F]PFHxA/serum mixture after the precipitation of the serum proteins. The origin is marked with a blue line.

cartridges by washing the retained fluoride off with 25 mM NH_4OAc (pH 4.5), while still retaining PFAS.[6,9] This nonradioactive method for purification should transfer easily to the radioactive samples. However, before our attempts to purify the products, the retention of free $^{18}F^-$ on the WAX cartridges was assessed. Fluorine 18 was diluted in water and loaded directly onto a freshly prepared WAX cartridge. On average, 31 ± 8% (n = 3) of the loaded activity was trapped onto the cartridge. After the cartridge was washed with 4 mL of 25 mM NH_4OAc (pH 4.5), it was determined that <2% of the initially loaded activity was retained. We concluded that this was an acceptable method for purification of the [^{18}F]PFAS compounds from any unreacted $^{18}F^-$ remaining in the reaction mixture.

On average, [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA were radiolabeled and isolated in decay-corrected yields of 13 ± 9% (n = 8), 31 ± 9% (n = 3), and 12 ± 3% (n = 2), respectively. The purities of the compounds were examined via iTLC. Figure 1 shows iTLC comparisons between [^{18}F]PFHxA in 0.1% NH_4OH in methanol (A) and free $^{18}F^-$ in the K_2CO_3 solution (aqueous) (B). All three [^{18}F]PFAS migrate with the mobile phase to ~85 mm (R_f = 1.0), and unreacted $^{18}F^-$ stays at the origin, ~30 mm (R_f = 0). Assuming all of the nonradioactive PFAS was co-eluted with the radiolabeled product during purification, the estimated average specific activities of the final products were calculated to be 60 ± 10 MBq/μmol for [^{18}F]PFOA, 12 ± 4 MBq/μmol for [^{18}F]PFHxA, and 3.4 ± 0.4 MBq/μmol for [^{18}F]PFBA. The labeling method has proven to be quite robust and could likely be applied in the future for the radiolabeling of any per- or polyfluorinated compound.

Stability Studies. While it is known that nonradiolabled PFAS are stable in a variety of environments,[14,15] the stability of the radiofluorinated compounds needed to be assessed. On average, [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA were <10% defluorinated over a 4 h period under all conditions, as determined by iTLC analysis. To assess the in vitro stabilities of the compounds in biologically relevant systems, [^{18}F]PFAS were incubated in mouse serum, human serum, and rat serum. The literature has shown that each of the compounds has a different biological half-life in vivo in these systems.[16] Initially, the serum stability studies were performed in the same manner as the stability tests in saline and 0.1% NH_4OH, by removing aliquots and analyzing them directly using iTLC. However, the results were convoluted (see Figure 2A), as the protein peak observed at 30 mm had the same R_f as free $^{18}F^-$. Thus, a second method of analysis (via protein precipitation) was used to properly determine the true serum stability of each compound. Figure 2B illustrates the iTLC results of the acetonitrile supernatant after the proteins were precipitated and removed from the mixture. Both the supernatant and the protein pellet were assessed for radioactivity. The small peak observed at 30 mm is most likely free fluorine; however, the possibility of protein-bound [^{18}F]PFHxA cannot be ruled out as this precipitation method does not guarantee complete precipitation of the proteins.[17] The results of the serum binding studies with [^{18}F]PFAS in mouse serum are presented in Figure 3. Results of the three [^{18}F]PFAS in rat and human sera are included in the Supporting Information.

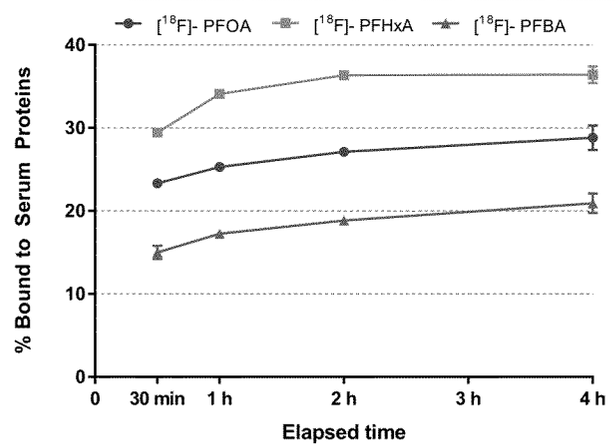

Figure 3. Results of the serum stability studies with [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA in mouse serum. The data were calculated as the percent of total activity bound to the serum proteins.

Biodistribution Studies in Normal Mice. The biodistribution studies of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA were performed in healthy mice. Fifteen different organs and tissues were dissected and assayed 4 h postinjection using a gamma counter to quantitatively determine the uptake of the tracer. This figure does not represent all the activity injected. Urine and feces were not collected in this study but would account for the remainder of activity not represented. A

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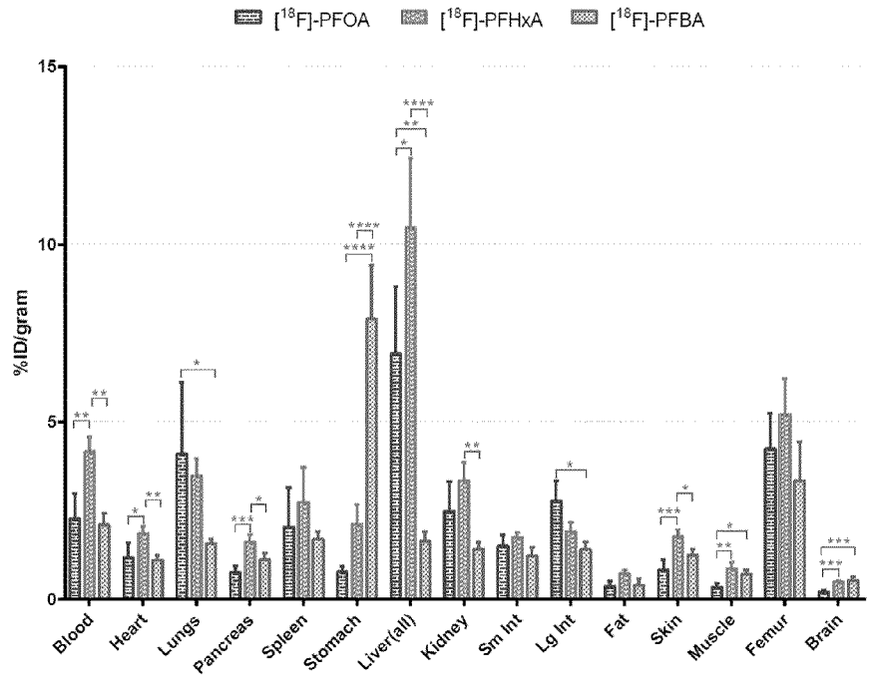

Figure 4. Complete biodistribution results of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA in healthy mice, 4 h postinjection. The results are presented as the percent of injected dose per gram of tissue. Error bars are indicative of the standard deviation (n = 4). Statistical analysis was performed to determine statistical differences (p values). Data are presented in Table S1. *p < 0.05. **p < 0.008. ***p < 0.0006. ****p < 0.0001.

comparison of the biodistribution results of each tracer is seen in Figure 4.

Each of the tracers exhibited some degree of uptake in all the organs and tissues of interest that were tested, including the brain. The highest observed uptake of [^{18}F]PFOA was in the liver, with 7 ± 2%ID/g; however, similar amounts were observed in the femur (4 ± 1%ID/g) and lungs (4 ± 2%ID/g). Uptake of [^{18}F]PFHxA was highest in the liver and femur with 10 ± 2 and 5 ± 1%ID/g, respectively. It should be stated that free $^{18}F^-$ is known to accumulate in bone;[18−20] however, it is not known at this time if the compounds were accumulating in the bone marrow or bone matrix.[21,22] Studies are ongoing to determine the "state", free $^{18}F^-$ or intact [^{18}F]PFAS, and location of the PFAS compounds in the femur. Unlike those of the other two compounds, the highest uptake of [^{18}F]PFBA was found in the stomach, with 8 ± 2%ID/g. The liver showed a 4−6-fold decrease in uptake with just 1.7 ± 0.7%ID/g when compared to those of [^{18}F]PFOA and [^{18}F]PFHxA.

Implications. The method development described here describes the first successful radiolabeling of three forms of PFAS with fluorine 18, its apparent stability in biological media, and its preliminary biodistribution in mice. It appears likely that any PFAS that can be synthesized and isolated could be radiolabeled in such a manner and used to directly measure uptake and biodistribution kinetics in biological systems. Because ^{18}F has a relatively short half-life, it is less likely to be useful for elimination kinetic studies, but it does open the possibility of directly measuring uptake in human subject volunteers, because trace amounts of the compounds can be easily measured and the radioactivity short-lived. The only previous human biodistribution study, by Perez et al.,[4] used cadavers, although there are some similarities found in the mouse biodistribution reported here. Similarly, this novel tool for studying PFAS behavior could be used in environmental remediation studies to measure the fate of radiolabeled compounds in environmental treatment systems. Further studies are needed for different PFAS compounds as well as different biological and environmental systems to assess the full impact of this novel radiosynthetic method.

ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.7b00042.

Radiosynthesis and isolation of [^{18}F]PFAS, serum stability study in rat and human serum (Figure S1), and biodistribution results of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA in healthy mice (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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